EUROPEAN PATENT APPLICATION

The microorganism(s) has (have) been deposited with Fermentation Research Institute under number(s) FERM BP-3254, FERM BP-3253 and FERM BP-2287.

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㉔ Structural gene of membrane-bound alcohol dehydrogenase complex,plasmid containing the same and transformed acetic acid bacteria.

㉗ There is provided a structural gene of membrane-bound alcohol dehydrogenase complex having a molecular size of about 7.0 kilo base which is derived from a microorganism belonging to the genus Acetobacter represented by Acetobacter altoacetigenes and shown by the nucleotide sequence of Fig. 3 and Fig. 4. This enzyme increases the efficiency of acetic acid fermentation and may be effectively utilized for quantitative determination of alcohol.

EP 0 448 969 A2

The present invention relates to a structural gene of membrane-bound alcohol dehydrogenase complex derived from a microorganism belonging to the genus Acetobacter, and a plasmid containing the same as well as its utilization.

A membrane-bound alcohol dehydrogenase produced by a microorganism belonging to the genus
5 Acetobacter is an enzyme which oxidizes an alcohol into the corresponding acid . The enzyme takes a part in oxidative fermentation of acetic acid fermentation for producing acetic acid from ethanol, and is also utilized for quantitative determination of alcohol; the enzyme is useful from an industrial viewpoint.

Heretofore the membrane-bound alcohol dehydrogenase (hereafter simply referred to as ADH) has been obtained by culturing a microorganism belonging to the genus Acetobacter or the genus Gluconobac-
10 ter, extracting and purifying from the cultured cells and has been utilized (Agricultural and Biological Chemistry, 42, 2045, 1978; ibid., 42, 2331, 1978).

For purification of this enzyme, however, fractionation by complicated column chromatography was required so that it was difficult to prepare the enzyme in large quantities. In addition, the enzyme is unstable and cannot be stored over a long period of time, which has been a problem in its application.

15 In order to solve these problems, it is considered to harvest mutants having an enhanced enzyme content in the cells by a mutation treatment. However, there is no report yet that any mutant having a sufficient enzyme content was harvested. It is also considered to achieve the object by cloning a gene of the enzyme and increasing the copy number of the enzyme gene or enhancing an expression degree, through genetic engineering technology. For this attempt, ADH gene of Acetobacter aceti K6033 strain has
20 been cloned and its nucleotide sequence has been determined (Journal of Bacteriology, 171, 3115, 1989). This study is expected to be effective for improving the productivity of the enzyme by genetic engineering technology. In actuality, however, even though a plasmid carrying the enzyme gene is introduced into a host of acetic acid bacteria, the enzyme activity is not improved more than the activity inherently possessed by the host and such technique is not practical.

25 This is believed to be because the cloned gene would be composed only of subunits having a larger molecular weight out of the subunits constructing ADH. Any conventional ADH is purified in the form of a complex with cytochrome c from acetic acid bacteria. Matsushita et al. reported that the activity of ADH was affected depending upon the quantity of cytochrome c and cytochrome c was not present merely as an impurity but took a part in expressing the enzyme activity (Agricultural and Biological Chemistry, 53, 2895,
30 1989). For this reason, it was necessary to increase the subunits having a large molecular weight and at the same time, increase the amount of the subunits of cytochrome c.

Furthermore, properties of the enzyme in the cloned Acetobacter aceti K6033 strain were not studied and utility of the enzyme of K6033 strain is unclear.

In order to solve the foregoing problems, the present inventors have brought attention to ADH produced
35 by a series of microorganisms belonging to the genus Acetobacter represented by Acetobacter altoacetigenes, which are already known to have enzymatically excellent properties, and have succeeded in cloning the structural gene of two proteins (subunits) constructing ADH and in carrying the structural gene on a plasmid.

Furthermore, the present inventors have found that by using the plasmid carrying the cloned gene, the
40 content of this enzyme in the cells can be increased, ADH can be readily extracted and purified, and the efficiency of acetic acid fermentation can be improved. The present invention has thus been accomplished.

The present invention relates to a structural gene of ADH complex which is derived from a microorganism belonging to the genus Acetobacter, has the restriction map given in Fig. 1 and has a length of about 7.0 kilobase pairs (kb). The invention further relates to a plasmid carrying the gene as well as a
45 microorganism belonging to the genus Acetobacter or the genus Gluconobacter transformed with the plasmid. The present invention further relates to a structural gene of a protein constituting an ADH complex, which is represented by the nucleotide sequence shown in Fig. 3 and has a molecular weight of about 72,000, and a plasmid carrying the gene as well as a microorganism belonging to the genus Acetobacter or the genus Gluconobacter transformed with the plasmid. The present invention also relates to a structural
50 gene of a protein constituting an ADH complex, which is represented by the nucleotide sequence shown in Fig. 4 and has a molecular weight of about 44,000, and a plasmid carrying the gene as well as a microorganism belonging to the genus Acetobacter or the genus Gluconobacter transformed with the plasmid.

Fig. 1 shows the restriction map of the structural gene of a membrane-bound alcohol dehydrogenase
55 complex isolated using Pst I.

Fig. 2 shows the restriction enzyme map of the structural gene of a protein having a molecular weight of about 72,000 which constitutes a membrane-bound alcohol dehydrogenase complex isolated using Sma I.

Fig. 3: the upper lines show the nucleotide sequence of the structural gene of a protein having a molecular weight of about 72,000 which constitutes a membrane-bound alcohol dehydrogenase complex isolated using Sma I, and the lower lines show the amino acid sequence determined from the nucleotide sequence of the structural gene of a membrane-bound alcohol dehydrogenase complex.

Fig. 4: the upper lines show the nucleotide sequence of the structural gene of a protein having a molecular weight of about 44,000 which constitutes a membrane-bound alcohol dehydrogenase complex isolated using Pst I, and the lower lines show the amino acid sequence determined from the nucleotide sequence of the structural gene of a membrane-bound alcohol dehydrogenase complex. Abbreviations in the amino acid sequences have the following meaning:

Met	methionine	Ala	alanine
Arg	arginine	Asn	asparagine
Asp	aspartic acid	Cys	cystein
Gln	glutamine	Glu	glutamic acid
Gly	glycine	His	histidine
Ile	isoleucine	Leu	leucine
Lys	lysine	Phe	phenylalanine
Pro	proline	Ser	serine
Thr	threonine	Trp	tryptophan
Tyr	tyrosine	Val	valine

The membrane-bound alcohol dehydrogenase complex in the present invention refers to a novel alcohol dehydrogenase complex having excellent stability which is described in Japanese Patent Application Laid-Open No. 63-12278 and composed of two proteins having molecular weights of about 72,000 and about 44,000. This enzyme is produced by a series of microorganisms belonging to the genus Acetobacter represented by Acetobacter altoacetigenes MH-24 (FERM BP-491).

The gene fragment containing the structural gene of the enzyme can be cloned from the total DNA which the microorganism belonging to the genus Acetobacter capable of producing this enzyme carries.

The total DNA may be prepared by, for example, the method disclosed in Japanese Patent Application Laid-Open No. 60-9489. The gene fragment containing the structural gene of the ADH complex may be cloned from the total DNA by, for example, the procedures shown in Example 1, that is, determining a part of the amino acid sequence of this enzyme, preparing synthetic DNA corresponding to the determined amino acid sequence and selecting a clone having the desired gene utilizing the synthetic DNA as a probe; etc. The amino acid sequence may be determined as follows: after the alcohol dehydrogenase complex purified by the method disclosed in Japanese Patent Application Laid-Open No. 63-12278 is separated into two subunits by SDS-polyacrylamide gel electrophoresis, the protein corresponding to each subunit is extracted from the gel in a conventional manner such as electric dialysis, etc. The extracted protein is used for determination of amino acid sequence at the amino terminus as it is. Alternatively, after the protein is cleaved with CNBr or with a protease (peptidase) having a high specificity, the cleavage product is fractionated by gel filtration, etc. and the resulting fraction is used for determination of amino acid sequence at the amino terminus in a conventional manner using an amino acid sequencer, etc. Synthesis of DNA corresponding to the thus determined amino acid sequence may be carried out in a conventional manner.

An antibody to the enzyme may be prepared by separating into two subunits the alcohol dehydrogenase complex purified by the method disclosed in Japanese Patent Application Laid-Open No. 63-12278 by SDS-polyacrylamide gel electrophoresis, extracting the protein corresponding to each subunit from the gel in a conventional manner such as electric dialysis, etc. and using the extracted protein as an antigen. Specifically, anti-ADH antibody may be obtained by, for example, the method described in

"Methods in Enzymology", 73, 46 (1981). About 2 weeks after the first immunization, a second immunization is made and in a month to a month and a half, the production of the antibody specific to ADH is observed. This antibody may be further purified either through purification by ammonium sulfate fractionation, etc. or by ion exchange chromatography. In the case that the antibody is used to clone the gene, it may also be possible to use appropriately diluted serum.

On the other hand, the cleavage product of the total DNA with an appropriate restriction enzyme is ligated with the cleavage product of an appropriate vector with a restriction enzyme capable of ligating with the total DNA using T4 DNA ligase. The ligation product is transformed to E. coli host. Examples of the vector used in this case include vectors of E. coli generally used, such as pBR322, pUC18, pUC19, and the like.

Transformation of E. coli may be conducted in a conventional manner. Detection of a strain bearing the desired gene can be made by preparing synthetic DNA based on the amino acid sequence previously determined using the purified enzyme and performing conventional colony hybridization using the synthetic DNA as a probe, whereby a strain reactive with the probe is selected.

Also where antigen-antibody reaction is utilized, a strain carrying the desired gene may be detected by a method similar to, e.g., Gene, 37, 267 (1985). That is, the lysate of the resulting transformants is reacted with the antibody and a strain showing a specific reaction may be selected.

The strain selected by the procedures described above may have a plasmid carrying the gene fragment having the entire length of the desired gene but may sometimes carry merely a part of the gene.

Where the strain has merely a part of the gene, the entire length of the gene may be obtained by using as a probe the gene already obtained and isolating a fraction showing homology to the probe by Southern hybridization, etc.

The nucleotide sequence of the resulting gene may be determined in a conventional manner, for example, by the dideoxy method using M13 phage.

In order to produce the ADH complex or the proteins constructing the ADH complex using the thus isolated gene fragment containing the structural gene of the ADH complex, in general, it is necessary to ligate the gene fragment carrying the enzyme gene with a gene having a promoter activity functioning in a host in the form of capable of expression. As the promoter used to produce the ADH complex proteins in a microorganism belonging to the genus Acetobacter or the genus Gluconobacter, there may be used a promoter inherently possessed by the ADH complex gene and there may also be used an acetic acid bacteria-derived gene having other promoter activity and a promoter of E. coli capable of expressing in acetic acid bacteria. As the E. coli promoter, there may be used promoters of ampicillin-resistant gene of E. coli plasmid pBR322, kanamycin-resistant gene of E. coli plasmid pACYC177, chloramphenicol-resistant gene of E. coli plasmid pACYC184, B-galactosidase gene of E. coli, etc. Where the ADH complex is produced in an excess amount to affect growth or the like of the host, it is necessary to choose an appropriate promoter for controlling an expression amount of the gene. Where the gene is expressed, formation of a protein having a size different from the molecular weight of the gene is sometimes observed. This is because the protein is produced in a host in the form of a fused protein in which other protein is fused. However, if the fused protein is produced in such a form that its enzyme activity can be expressed, there would be no problem.

As the vector for carrying the gene fragment containing the structural gene of the ADH complex in acetic acid bacteria, there may be utilized, for example, pTA5001(A) and pTA5001(B) disclosed in Japanese Patent Application Laid-Open No. 60-9488; wide host range vectors RP4::Mu, RP4, pRK2013, RSF1010 etc. which can be introduced into acetic acid bacteria.

For expression of the activity of ADH, it is necessary that the two proteins constituting the ADH complex be produced efficiently with good balance, as shown in the EXAMPLES. In general, the gene fragment containing the structural gene of the ADH complex is used as it is and the two proteins may be expressed on the same level. Depending upon acetic acid bacteria, however, either protein is not sufficiently possessed in some occasion. In this case, it is required that the gene encoding the two proteins are independently prepared and the genes having a promoter activity used to express the genes are selected to be a suitable expression amount, respectively. For controlling the expression amount, it may also be possible to use different vectors in the two genes and utilize a difference in the copy number of the vectors in acetic acid bacteria.

As stated above, the plasmid containing the structural gene of the ADH complex can be isolated. After transformation, the gene is expressed, whereby the protein constituting the ADH complex can be produced in a marked quantity.

As the host for producing the ADH complex, microorganisms such as E. coli, Bacillus subtilis, etc. on which genetic engineering technique has been established may be used. However, it is more advantageous

to use acetic acid bacteria which inherently possess the ability of producing the ADH complex, namely, the microorganisms belonging to the genus Acetobacter or the genus Gluconobacter.

ADH has pyrroloquinoline quinone (PQQ) as its prosthetic group. In order to produce an activated enzyme, PQQ may be supplemented to a medium, etc. to produce the ADH complex protein. However, as

5 is described in Agricultural & Biological Chemistry, 48, 561 (1984), the ability of E. coli or S. subtilis for synthesizing PQQ is poor and it has been revealed that the synthesizing ability of acetic acid bacteria is high. It is thus advantageous for the host to have the ability for synthesizing PQQ.

Further in acetic acid fermentation, ADH participates in the reaction of oxidizing ethanol to acetaldehyde. For this reason, by enhancing the content of the ADH complex in acetic acid bacteria, it can be

10 expected to make the acetic acid fermentation efficient. In this case, where ADH alone is expressed excessively, the concentration of acetaldehyde, which is the oxidation product of ethanol, increases so that acetic acid bacteria are damaged by strongly cytotoxic acetaldehyde. Therefore, it is necessary either to control the amount of the ADH complex gene expressed to the amount corresponding to the oxidising activity of acetaldehyde or to increase the amount of aldehyde dehydrogenase at the same time, using the

15 structural gene of the membrane-bound aldehyde dehydrogenase recited in Japanese Patent Application Laid-Open No. 63-52709 so as not to cause excessive accumulation of acetaldehyde.

[EXAMPLES]

20 The present invention is illustrated by the following examples.

EXAMPLE 1

[Determination of amino terminal amino acid sequence and preparation of synthetic probe]

25

Acetobacter altoacetigenes MH-24 (FERM BP-491) strain was shaking cultured at 30°C in medium composed with 3% of glucose, 4% (V/V) of ethanol, 6% (V/V) of acetic acid, 0.5% of yeast extract (manufactured by Daigo Nutrient Chemistry Co., Ltd.) and 0.2% of polypeptone (manufactued by Daigo Nutrient Chemistry Co., Ltd.).

30 After the incubation, the cells were harvested by centrifugation and 10 mg of the ADH complex was then obtained in a conventional manner (the method disclosed in Japanese Patent Application Laid-Open No. 63-12278). This complex was subjected to SDS-polyacrylamide gel electrophoresis to separate the protein having a molecular weight of about 72,000 and the protein having a molecular weight of about 44,000. Then, the protein of 72,000 was eluted from the gel in a conventional manner and provided as a

35 sample for the following experiment.

After 1 mg of the sample obtained was cleaved with lysyl endopeptidase (manufactured by Wako Pure Chemicals, Inc.), the cleavage product was fractionated by HPLC LC-4A manufactured by Shimadzu Seisakusho Co., Ltd. As a column Senshu Pak. VP-304-1251 (4.6 ø × 250 mm) was used and the elution was performed at a flow rate of 1 ml/min and at room temperature by linear gradient of acetonitrile-water

40 (containing 0.1% trifluoroacetic acid) of 0 to 55%. By monitoring at absorbance of 220 nm, 11 peaks were noted. From the earlier order of elution, the second, ninth and eleventh peaks were fractionated. About 0.5 mg of the fractionated product was applied to amino acid sequencer Model 470A manufactured by Applied Biosystems Inc. to determine the amino terminal amino acid sequence. The results reveal that the sequence of the peptide eluted in the ninth order was:

45 Thr-Gly-Leu-Val-Tyr-Ile-Pro-Ala-Gln-Gln-Val-Pro-Phe-Leu-Tyr-Thr-Asn-Gln-Val-Gly-Gly-Phe-Tyr-Pro-His-Pro-Asp; and that the sequence of the peptide eluted in the eleventh order was: Leu-Ala-Trp-Tyr-Leu-Asp-Leu-Asp-Thr-Asn-Arg-Gly-Gln-Glu-Gly-Thr-Pro-Leu. Furthermore, the sequence of the peptide eluted in the second order was: Asn-Tyr-Val-Tyr-Val-Asn-Trp-Ala-Ser-Gly-Leu-Asp-Pro.

The protein having a molecular weight of 72,000 which was not treated with lysyl endopeptidase was

50 analyzed with an amino acid sequencer. The amino terminal amino acid sequence was Asp-Asp-Gly-Gln-Gly. DNA corresponding to the amino acid sequence was synthesized with DNA synthesizer 381A manufactured by Applied Biosystems Inc., based on the two sequences Tyr-Ile-Pro-Ala-Gln-Gln-Val (Sequence 1) and Val-Ile-Ile-Gly-Asn-Gly (Sequence 2) in the amino acid sequence of the peptide eluted in the ninth order and a part of the amino acid sequence, Try-Val-Tyr-Val-Asn-Trp-Ala (sequence 3), in the

55 peptide eluted in the second order, taking utilization of codon into account.

For Sequence 1, Probe 1 a 64-fold degenerate 20-mer was synthesized:

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          T    T

    TA  AT  CCNGCNCAGCAGCAGG

          C    C
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For Sequence 2, Probe 2, a 128-fold degenerate 17-mer was synthesized:

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              T    T      T

    GTNAT  AT  GGNAA  GG

              C    C      C
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For Sequence 3, Probe 3, a 128-fold degenerate 20-mer was synthesized:

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         A       A   A A

    GCCCA  TTNAC  TAN  C  TA

         G       G   G G
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This sequence was derived from the complementary strand.

Cloning of the structural gene of protein having a molecular weight of about 72,000 which constructs the ADH complex

From the cells of Acetobacter altoacetigenes MH-24 strain which had been obtained by culturing as described above, the total DNA was prepared in a conventional manner (the method disclosed in Japanese Patent Application Laid-Open No. 60-9489). After the total DNA was cleaved with a restriction enzyme, Pst I or Sma I (manufactured by Takara Shuzo Co., Ltd.), the product was ligated with E. coli vector pUC18 (manufactured by Takara Shuzo Co., Ltd.) which was cleaved with Pst I or Sma I, and thereafter dephosphorylated with bacterial alkaline phosphatase (manufactured by Takara Shuzo Co., Ltd.), using T4 DNA ligase (manufactured by Takara Shuzo Co., Ltd.). After the ligation mixture was transformed to the host E. coli JM 109 by the method of Hanahan ["DNA Cloning", 1, 109, IRL Press (1985)], transformants were selected in LB agar medium ("A Manual for Genetic Engineering", page 201, Cold Spring Harbor Laboratory, 1980) containing ampicillin in a concentration of 30 μg/ml.

With respect to about 5,000 recombinants obtained, colonies which hybridized with Probe 2 and Probe 3 described above were detected according to the colony hybridization technique ("A Manual for Genetic Engineering", page 312, Cold Spring Harbor Laboratory, 1980) using the two probes. In Pst I, three (3) clones hybridized with Probes 2 and 3,and in Sma I, two (2) clones hybridized with the probes. Furthermore, these 5 clones all hybridized also with Probe 1.

Analysis with restriction enzyme reveals that all of the 3 clones obtained using Pst I had the same fragment of about 7.0 kilo base at the Pst I site of pUC18. Further,in the case of Sma I, the clones had the same fragment of about 4.5 kilo base. The fragment of about 7.0 kilo base obtained with Pst I had a portion of about 4.1 kilo base in common to the fragment of about 4.5 kilo base. The plasmid (chimeric plasmid composed of pUC18 and the insert fragment of about 7.0 kilo base, named pADHP1) possessed by 1 clone obtained using Pst I was transformed in E. coli JM 109 and has been deposited in the Fermentation Research Institute of the Agency of Industrial Science and Technology of Japan under the name of E. coli ADHP-1 as [FERM BP-3254 (FERM P-11278)]. The restriction enzyme map of the insert fragment of about 7.0 kilo base was prepared in a conventional manner, which is as shown in Fig. 1. Furthermore, the plasmid (chimeric plasmid composed of pUC18 and the insert fragment of about 4.5 kilo base, named pADHS1) possessed by 1 clone obtained using Sma I was transformed in E. coli JM 109 and has been deposited in the Fermentation Research Institute of the Agency of Industrial Science and Technology of Japan under the name of E. coli ADHS-1 as [FERM BP-3253 (FERM P-11201)]. The restriction enzyme map of the insert

fragment of about 4.5 kilo base was prepared in a conventional manner, which is as shown in Fig. 2.

With respect to the insert fragment of pADHS1, its nucleotide sequence was determined by the dideoxy method [Methods in Enzymology, 10, 20, Academic Press, New York, 1983] using M13 phage.

Based on the thus determined nucleotide sequence, an open-reading frame was identified. The open-reading frame encoding 738 amino acid residues (molecular weight 80839), composed of 2214 bases and translated from the ATG initiation codon as shown in Fig. 3 was identified in the portion common to the Sma I fragment having a size of about 4.5 kilo base and the Pst I fragment having a size of about 7.0 kilo base (the amino acid sequence determined from the nucleotide sequence of Fig. 3 is shown in Fig. 3, lines below the nucleotide sequence). The polypeptide encoded by the nucleotide sequence of Fig. 3 coincides with the protein having a molecular weight of about 72,000 which constitutes the membrane-bound alcohol dehydrogenase complex of the present invention. This is confirmed by the fact that when the amino acid sequence of the purified protein having a molecular weight of about 72,000, which constructs the membrane-bound alcohol dehydrogenase complex of the present invention, was determined by the method described above, the sequence fully coincident with the amino terminal amino acid sequences of the 3 peptides of the lysyl endopeptidase cleavage products was found. That is, the sequence of 27 amino acids of the peptide eluted in the ninth order coincided with the sequence of 27 amino acids following 442 amino acid from the amino terminus deduced from the nucleotide sequence. Furthermore, the sequence of 18 amino acids of the peptide eluted in the eleventh order coincided with the sequence of 18 amino acids following 84 amino acid deduced from the nucleotide sequence. The amino terminal amino acid sequence of the peptide eluted in the second order coincided with the sequence of 13 amino acids following 389 amino acid deduced from the nucleotide sequence.

Furthermore, the amino terminal sequence (Asp-Asp-Gly-Gln-Gly) of the purified protein completely coincided with the amino acid sequence following the 36th counted from the amino terminus which is deduced from the nucleotide sequence. It is thus assumed that the amino acid sequence up to the 35th from the amino terminus deduced from the nucleotide sequence would be the region which participates in secretion of the protein having a molecular weight of about 72,000. Acetobacter aceti K6033 strain had homology of about 77% to ADH gene in the amino acid sequence.

Preparation of anti-ADH antibody

Acetobacter altoacetigenes MH-24 (FERM BP-491) strain was shakingly cultured at 30° C in medium composed with 3% of glucose, 4% (V/V) of ethanol, 6% (V/V) of acetic acid, 0.5% of yeast extract (manufactured by Daigo Nutrient Chemistry Co., Ltd.) and 0.2% of polypeptone (manufactured by Daigo Nutrient Chemistry Co., Ltd.). After the incubation, the cells were harvested by centrifugation and 4 mg of the ADH complex was then obtained in a conventional manner (the method disclosed in Japanese Patent Application Laid-Open No. 63-12278). This complex was subjected to SDS-polyacrylamide gel electrophoresis to separate the protein having a molecular weight of about 72,000 and the protein having a molecular weight of about 44,000. The respective proteins were eluted from the gel in a conventional manner and provided as samples for the following experiment.

Each 0.1 mg of the samples obtained was subcutaneously injected to rabbit together with complete Freund's adjuvant, and 0.1 mg of each sample was further injected after about 2 weeks. One month after the first injection, rabbit blood was withdrawn from the ear and centrifuged. The reactivity of the thus obtained serum with the two proteins was examined, whereby precipitation was noted. Further after SDS-polyacrylamide gel electrophoresis, its specificity was examined by Western blotting, using the cell-free extract of Acetobacter altoacetigenes MH-24 and E. coli JM 109. Reactivity with proteins other than the objective protein was not appreciable but the antibody having high specificity was produced.

Cloning of gene containing the full length of the structural gene of ADH complex

The Pst I fragment having a size of about 7.0 kilo base containing the structural gene of the protein having a molecular weight of 72,000, which constructed the ADH complex, obtained by the procedures described above and the Sma I fragment having a size of about 4.5 kilo base were ligated at the Pst I site or Sma I site of E. coli vector pUC18, respectively, in a conventional manner. The ligated chimeric plasmid was transformed to E. coli JM 109 in a conventional manner to give transformants carrying the chimeric plasmids. From the transformants, the plasmids were prepared in a conventional manner followed by analysis with restriction enzymes.

By the analysis with restriction enzymes, selection was made for chimeric plasmids in which the Pst I fragment or the Sma I fragment was inserted in such a way that the transcription direction of the lac

promoter of E. coli vector pUC18 was the same as the transcription direction of the structural gene of the protein having a molecular weight of about 72,000, which constructed the ADH complex. The transformants carrying these plasmids were cultured at 37°C for 8 hours in LB medium containing 30 μg/ml of ampicillin and 1 mM of isopropyl-β-thiogalactopyranoside (IPTG). The cells were sonicated, and the resulting
5 homogenate was subjected to SDS-polyacrylamide gel electrophoresis. A molecular weight of the protein specifically reacting with the antibody was determined using an antibody capable of specifically reacting with the two proteins which constructed the ADH complex described above, according to the Western blotting method (Annal. Biochem., 12, 195 (1981)). When detection was made using the antibody to the protein having a molecular weight of about 72,000, the reaction with the protein having a molecular weight
10 of about 72,000 was noted both in the case of carrying the Pst I fragment and in the case of carrying the Sma I fragment. In the transformant carrying vector pUC18 alone which was used for control, no protein capable of reacting with the antibody was detected. By the foregoing, it was confirmed that the structural gene of the protein having a molecular weight of about 72,000 was present on the Pst I fragment and on the Sma I fragment.

15   On the other hand, detection was made using the antibody to the protein having a molecular weight of about 44,000. In the transformant carrying only vector pUC18 that was used for control, no protein capable of reacting with the antibody was noted. However, in the transformant carrying the plasmid into which the Sma I fragment had been inserted, the reaction with the protein having a molecular weight of about 24,000 was noted. Further in the transformant carrying the plasmid into which the Pst I fragment had been inserted,
20 the reaction with the protein having a molecular weight of about 44,000 was noted. To the contrary, in the cells cultured in liquid medium containing no IPTG, the protein having a molecular weight of about 44,000 and capable of reacting with the antibody was not detected.

These results indicate that the structural gene encoding the protein having a molecular weight of about 44,000, which is cytochrome c, is present on the Pst I fragment and the direction of its transcription is the
25 same as that of the protein having a molecular weight of about 72,000. From the fact that the molecular weight is about 44,000, it is also assumed that the region of the structural gene necessary for encoding this protein would be about 1.2 kilo base. Taking the size of the protein capable of reacting with the antibody into account, it is assumed that the structural gene of cytochrome c having a molecular weight of about 44,000 would be present immediately downstream the structural gene of the protein having a molecular
30 weight of about 72,000 and transcribed and expressed in one unit.

Based on the foregoing results, it was confirmed that the structural genes of the protein having a molecular weight of about 72,000 and the protein having a molecular weight of about 44,000 are present on the gene fragment cleaved with Pst I in the restriction enzyme map shown in Fig. 1.

35 EXAMPLE 2

Transformation of the gene fragment containing the structural gene of ADH complex into acetic acid bacteria host

40   Chimeric plasmid pADHS1 of the Sma I fragment (about 4.5 kilo base) containing the structural gene of the protein having a molecular weight of about 72,000, which constructed the ADH complex isolated in EXAMPLE 1 was extracted from E. coli ADHS-1 in a conventional manner to give purified DNA. After 1 μg of this DNA was cleaved with Sac I, the cleavage end was rendered blunt with T4 DNA polymerase. On the other hand, plasmid named pTA5001 was prepared from Acetobacter aceti No. 1023 [FERM BP-2287
45 (FERM P-7122)] according to the method described in Agricultural and Biological Chemistry, 49, 1011 (1985) (pTA5001 is described in Agricultural and Biological Chemistry, 49, 1011 (1985). pTA5001 is a mixture of two plasmids of pTA5001A having a length of 23.5 kilo base and pTA5001B having a length of 23 kilo base.). After 5 μg of this plasmid DNA werecleaved with Xho I, the cleavage end was rendered blunt with T4 DNA polymerase.

50   The cleaved DNAs of pADHS1 and pTA5001 prepared as described above were ligated with each other using T4 DNA ligase to give the ligation product. Thereafter, the product was transformed in ADH activity-deleted mutant 10-80 according to the method described in Agricultural and Biological Chemistry, 49, 2091 (1985). The transformants were selected in YPG agar medium (3% of glucose, 0.5% of yeast extract, 0.2% of polypeptide, 2% of agar, pH 6.5) containing 50 μg/ml of ampicillin. Plasmids of 10 ampicillin-resistant
55 strains grown in the selection medium were analyzed by a modified method of Agricultural and Biological Chemistry, 49, 2083 (1985). As the result, the size of the plasmids introduced were all about 31 kilo base. Analysis with restriction enzymes reveals that they were all chimeric plasmid of three components: pUC18, The Sma I fragment of 4.5 kilo base containing the structural gene of the protein having a molecular weight

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of about 72,000 which constructed the ADH complex, and pTA5001. This chimeric plasmid was named pMADHSI.

After pADHPI was cleaved with Sac I as in pADHSI, chimeric plasmid of plasmid pADHP1 isolated in EXAXPLE 1 and pTA500I was prepared in a manner similar to the case of pADHSI. The chimeric plasmid was transformed into mutant 10-80 of Acetobacter aceti No. 1023 to give the transformant carrying the chimeric plasmid (named pMADHPI).

Properties of acetic acid bacteria transformant

With respect to the two transformants of mutant 10-80 of Acetobacter aceti No. 1023 obtained above, enzyme activity of ADH was assayed. Firstly, ampicillin was added to YPG liquid medium (medium having a composition obtained by removing agar from YPG agar medium described above) in a concentration of 30 μg/ml followed by shaking-culture at 30°C for 36 hours. After culturing, the cells were harvested, suspended in McIlvaine buffer (pH 6.0) and homogenized with a French press. ADH activity in the supernatant obtained from the homogenate was measured by a method of Agricultural and Biological Chemistry, 49, 2045 (1978). At the same time, aldehyde dehydrogenase (ALDH) activity was also determined by a method of Agricultural and Biological Chemistry, 44, 503 (1980). These results are shown in Table 1.

Table 1

Strain	Chimera Plasmid Carried	Enzyme Activity (U/mg protein)	
		ADH	ALDH
No. 1023	none	0.28	0.94
10-80	none	0.01	0.85
10-80	pMADHS1	0.01	0.90
10-80	pMADHP1	0.40	1.00

Mutant 10-80 obtained from Acetobacter aceti No. 1023 is a strain which is specifically deleted of ADH activity. The transformant of this strain carrying plasmid pMADHSI containing the structural gene alone encoding the protein having a molecular weight of about 72,000 did not show ADH activity yet. On the other hand, in the transformant carrying plasmid pMADHPI concurrently containing the gene encoding the protein having a molecular weight of about 44,000, restoration of ADH activity was noted. From the results, it is shown that for expression of ADH activity, two proteins having a molecular weight of 72,00 and a molecular weight of 44,000 which construct the ADH complex are required.

It is also noted that the specific activity of the parent having no chimeric plasmid was 0.28 (unit/mg protein), whereas the the specific activity of transformant was 0.40, showing an increase of the activity by about 1.4 times.

As described above, the cell content of ADH having the activity can be increased by transforming acetic acid bacteria with the gene containing the structural gene of ADH complex.

EXAMPLE 3

Determination of nucleotide sequence of the structural gene of the protein having a molecular weight of about 44,000 which constructs the ADH complex

The results of EXAMPLE 1 reveal that the structural gene encoding the protein having a molecular weight of about 44,000 is present right downstream of the structural gene encoding the protein having a molecular weight of about 72,000. Therefore, the nucleotide sequence of an about 2.8 kilo base fragment containing the region downstream of the structural gene encoding a protein having a molecular weight of about 72,000 in the insert fragment of pADHPI, restriction enzyme map of which is shown in Fig. 1 (from the left Cla I site to the right BamH I site)

was determined by the dideoxy method (Methods in Enzymology, 10, 20, Academic Press, New York,

1983), using M13.

Based on the determined nucleotide sequence, the open-reading frame which could encode the protein having a molecular weight of about 44,000 downstream of the nucleotide sequence shown in Fig. 3 was analyzed and an open-reading frame which could encode a protein of 468 amino acid residues (molecular
5   weight of 49757) composed of 1404 bases starting with translation initiation codon ATG as shown in Fig. 4, was found (the amino acid sequence determined from the nucleotide sequence in Fig. 4 is shown in Fig. 4 below the nucleotide sequence). In order to confirm that the polypeptide having the amino acid sequence shown in Fig. 4 coincides with the protein having a molecular weight of about 44,000 which constitutes the membrane-bound alcohol dehydrogenase complex of the present invention, the protein having a molecular
10   weight of about 44,000 was isolated from the membrane-bound alcohol dehydrogenase complex. The protein was treated with Lysyl endopeptidase, the resulting cleavage product was fractionated and the amino terminal amino acid sequence of the resulting peptide was determined, in a manner similar to EXAMPLE 1. It is confirmed that the same amino acid sequence as that determined is present in the sequence shown in Fig. 4. That is, lysyl endopeptidase was acted on the protein having a molecular weight
15   of about 44,000 isolated in a manner similar to EXAMPLE 1. The resulting cleavage product was fractionated by HPLC in a manner similar to EXAMPLE 1. Among the eluted peptides, the first and fourth peptides were fractionated in the earlier order of elution. Using about 0.1 mg of the fractionated product, the amino acid sequence at the amino terminus was determined in a manner similar to EXAMPLE 1. As the result, the amino terminal amino acid sequence of the peptide firstly eluted was determined to be Asp-Phe-
20   Tyr-Pro-Ala-Pro and the amino terminal amino acid sequence of the peptide fourthly eluted was determined to be Ser-Leu-Ser-Ala-Glu-Glu.

These sequences coincided with the sequence after 169 and with the sequence after 390, from the amino terminus, in the amino acid sequence shown in the lower lines in Fig. 4. It was thus confirmed that the gene having the nucleotide sequence shown in Fig. 4 was the structural gene of the protein having a
25   molecular weight of about 44,000 which constituted the ADH complex.

According to the present invention, the structural gene of the ADH complex produced by a series of microorganisms belonging to the genus Acetobacter represented by Acetobacter altoacetigenes can be cloned and the structural gene can be successfully incorporated into a plasmid. Further by using acetic acid bacteria transformed by the plasmid, efficiency of acetic acid fermentation can be increased. Moreover, the
30   ADH complex can be readily extracted and purified from the acetic aced bacteria and this enzyme can be utilized for quantitative determination of alcohol.

While the invention has been described in detail and with reference to specific embodiments thereof, it is apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and the scope of the present invention.
35

1.   **SEQ ID NO: 1:**

40   **SEQUENCE TYPE:**   **nucleic acid**

        SEQUENCE LENGTH: **2214 bp**

        STRANDEDNESS: **double**

        MOLECULE TYPE: **genomic DNA**
45
        ORIGINAL SOURCE

        ORGANISM: **Acetobacter altoacetigenes MH-24**

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```
ATGATTTCTG CCGTTTTCGG AAAAAGACGT TCTCTGAGCA GAACGCTTAC AGCCGGAACG    60

ATATGTGCGG CTCTCATCTC CGGGTATGCC ACCATGGCAT CCGCAGATGA CGGGCAGGGC   120

GCCACGGGGG AAGCGATCAT CCATGCCGAT GATCACCCCG GTAACTGGAT GACCTATGGC   180

CGCACCTATT CTGACCAGCG CTACAGCCCG CTGGATCAGA TCAACCGTTC CAATGTCGGT   240

AACCTGAAGC TGGCCTGGTA TCTGGACCTT GATACCAACC GTGGCCAGGA AGGCACGCCC   300

CTGGTTATTG ATGGCGTCAT GTACGCCACC ACCAACTGGA GCATGATGAA AGCCGTCGAC   360

GCCGCAACCG GCAAGCTGCT GTGGTCCTAT GACCCGCGCG TGCCCGGCAA CATTGCCGAC   420

AAGGGCTGCT GTGACACGGT CAACCGTGGC GCGGCATACT GGAATGGCAA GGTCTATTTC   480

GGCACGTTCG ACGGTCGCCT GATCGCGCTG GACGCCAAGA CCGGCAAGCT GGTCTGGAGC   540

GTCAACACCA TTCCGCCCGA AGCGGAACTG GGCAAGCAGC GTTCCTATAC GGTTGACGGC   600

GCGCCCCGTA TCGCCAAGGG CCGCGTGATC ATCGGTAACG GTGGTTCCGA ATTCGGTGCC   660

CGTGGCTTCG TCAGCGCGTT CGATGCGGAA ACCGGCAAGG TCGACTGGCG CTTCTTCACG   720

GTTCCGAACC CCAAGAACGA ACCGGACGCT GCATCCGACA GCGTGCTGAT GAACAAGGCC   780

TACCAGACCT GGAGCCCGAC CGGCGCCTGG ACCCGCCAGG GTGGCGGCGG CACGGTATGG   840

GATTCCATCG TGTATGACCC CGTGGCCGAC CTGGTCTACC TGGGCGTTGG CAACGGTTCG   900

CCGTGGAACT ACAAGTACCG TTCCGAAGGC AAGGGCGACA ACCTGTTCCT GGGCAGCATC   960

GTCGCACTGA AGCCGGAAAC CGGCGAATAC GTCTGGCATT TCCAGGAAAC GCCGATGGAC  1020

CAGTGGGACT TCACCTCGGA CCAGCAGATC ATGACGCTTG ACCTGCCGAT CAATGGTGAA  1080

ACCCGCCACG TCATCGTCCA TGCGCGCAAG AACGGCTTCT TCTACATCAT CGATGCGAAG  1140

ACCGGTGAGT TCATCTCGGG CAAGAACTAC GTCTATGTGA ACTGGGCCAG CGGCCTTGAT  1200

CCCAAGACCG GCCGTCCGAT CTACAACCCC GATGCGCTCT ACACCCTTAC GGGCAAGGAA  1260

TGGTACGGCA TTCCGGGTGA CCTTGGCGGC CATAACTTCG CGGCCATGGC GTTCAGCCCC  1320

AAGACCGGGC TGGTCTATAT TCCGGCGCAG CAGGTTCCGT TCCTGTACAC CAATCAGGTC  1380

GGTGGCTTCA CGCCGCACCC CGACAGCTGG AACCTGGGTC TGGACATGAA CAAGGTCGGT  1440

ATTCCCGACT CGCCTGAAGC CAAGCAGGCC TTCGTGAAGG ACCTGAAGGG CTGGATCGTG  1500

GCCTGGGATC CGCAGAAGCA GGCTGAAGCA TGGCGCGTGG ACCACAAGGG GCCGTGGAAC  1560

GGCGGTATCC TGGCAACTGG CGGCGACCTG CTGTTCCAGG GCTTGGCGAA CGGCGAATTC  1620

CATGCCTATG ACGCGACGAA CGGTTCCGAC CTGTTCCACT TCGCGGCGGA CAGCGGCATC  1680
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ATCGCACCGC CTGTGACCTA CCTTGCCAAT GGCAAGCAGT ATGTTGCGGT TGAAGTGGGC    1740

TGGGGCGGCA TCTATCCGTT CTTCCTTGGT GGCCTGGCCC GTACCAGCGG CTGGACCGTC    1800

AACCACTCGC GCATCATTGC CTTCTCGCTC GATGGCAAGT CCGGCCCGCT GCCCAAGCAG    1860

AATGACCAGG GCTTCCTGCC CGTCAAGCCG CCGGCACAGT TCGACAGCAA GCGTACCGAT    1920

AACGGTTACT TCCAGTTCCA GACCTATTGC GCCGCCTGTC ATGGCGATAA CGCAGAAGGT    1980

GCCGGTGTGC TGCCTGACCT GCGCTGGTCC GGGTCCATCC GTCATGAGGA CGCGTTCTAC    2040

AATGTTGTCG GCCGCGGCGC GCTTACCGCC TACGGTATGG ATCGCTTGCA CGGTAACATG    2100

AACCCGACCG AGATTGAGGA CATCCGCCAG TTCCTGATCA AGCGTGCGAA CGAGACCTAT    2160

CAGAGGGAAG TTGATGCCCG GAAGAACGCT GACGGTATCC CCGAGCAGCT GCCG          2214
```

2.   <u>SEQ ID NO: 2:</u>

SEQUENCE TYPE:	nucleic acid
SEQUENCE LENGTH:	1404 bp
STRANDEDNESS:	double
MOLECULE TYPE:	genomic DNA
ORIGINAL SOURCE ORGANISM:	Acetobacter altoacetigenes MH-24

12

```
ATGATCAACA GACTTAAGGT GACATTCAGC GCGGCAGCGT TTAGTCTGCT GGCAGGGACG      60

GCATTGGCAC AGACGCCAGA TGCTGACTCC GCGCTGGTCC AGAAGGGGGC ATATGTCGCG     120

CGACTGGGTG ACTGCGTAGC ATGTCATACC GCTCTCCATG GACAGTCGTA CGCAGGCGGG     180

CTTGAAATCA AGAGCCCGAT CGGTACGATC TACTCCACGA ACATCACACC GGACCCGACC     240

TACGGTATCG GTCGCTACAC CTTCGCCGAA TTCGACGAAG CCGTGCGCCA TGGTATCCGC     300

AAGGACGGTT CCACGCTGTA TCCGGCCATG CCGTATCCCT CCTTCTCGCG CATGACGAAG     360

GAAGACATGC AGGCGCTGTA TGCGTACTTC ATGCATGGGG TGAAGCCGGT CGCGCAGCCG     420

GACAAGCAGC CGGACATCTC CTGGCCCTTG TCCATGCGCT GGCCGCTGGG CATCTGGCGC     480

ATGATGTTCT CGCCTTCGCC GAAGGACTTC ACGCCGGCGC CAGGCACGGA TCCTGAAATC     540

GCACGTGGCG ATTATCTGGT TACCGGCCCC GGGCATTGCG GTGCGTGTCA TACCCCGCGT     600

GGCTTCGCCA TGCAGGAAAA GGCGCTGGAC GCTGCCGGTG GTCCTGACTT CCTGTCCGGT     660

GGCGCACCGA TCGACAACTG GGTCGCGCCG AGCCTGCGCA ACGATCCTGT CGTTGGTCTG     720

GGCCGCTGGT CCGAGGATGA CATCTACACC TTCCTGAAGT CCGGCCGTAT CGACCACTCC     780

GCCGTGTTCG GTGGCATGGG CGATGTGGTG GCATGGAGCA CCCAGTACTT CACCGATGAC     840

GACCTGCACG CCATCGCGAA GTACCTGAAG AGCCTGCCGC CGGTGCCGCC GTCACAGGGC     900

AACTACACCT ACGATCCGTC CACCGCGAAC ATGCTGGCTT CGGGTAATAC CGCCAGCGTT     960

CCGGGTGCTG ATACGTATGT GAAGGAATGC GCCATCTGTC ACCGTAACGA CGGTGGTGGC    1020

GTGGCCCGCA TGTTCCCGCC GCTGGCTGGC AACCCGGTTG TCGTGACCGA GAACCCGACC    1080

TCGCTGGTGA ACGTGATTGC GCATGGTGGC GTGCTGCCGC CGAGCAACTG GGCACCGTCC    1140

GCAGTGGCAA TGCCGGGTTA CAGCAAGTCG CTGTCCGCCC AGCAGATTGC TGATGTGGTC    1200

AACTTCATCC GCACCAGCTG GGGCAACAAG GCGCCCGGCA CCGTTACGGC TGCGGATGTT    1260

ACCAAGCTGC GCGACACGGG CGCCCCGGTT TCCAGCTCTG GCTGGAACAG CGTGAGCAGC    1320

GGCTGGTCGG TCTTCCTGCC GCAGCCTTAC GGCTCGGGCT GGACGTTTGC CCCGCAGACG    1380

CACACCGGTC AGGACGCCGC ACAG                                          1404
```

3. <u>SEQ ID NO: 3:</u>

5

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 738

MOLECULE TYPE: protein

ORIGINAL SOURCE

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ORGANISM: Acetobacter altoacetigenes MH-24

FEATURES: The mature peptide consists of the amino
acids at positions 36 to 738.

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Met Ile Ser Ala Val Phe Gly Lys Arg Arg Ser Leu Ser Arg Thr Leu
 1           5                  10                  15

Thr Ala Gly Thr Ile Cys Ala Ala Leu Ile Ser Gly Tyr Ala Thr Met
               20              25              30

Ala Ser Ala Asp Asp Gly Gln Gly Ala Thr Gly Glu Ala Ile Ile His
           35              40                  45

Ala Asp Asp His Pro Gly Asn Trp Met Thr Tyr Gly Arg Thr Tyr Ser
           50              55                  60

Asp Gln Arg Tyr Ser Pro Leu Asp Gln Ile Asn Arg Ser Asn Val Gly
 65              70                  75                  80

Asn Leu Lys Leu Ala Trp Tyr Leu Asp Leu Asp Thr Asn Arg Gly Gln
                85                  90                  95

Glu Gly Thr Pro Leu Val Ile Asp Gly Val Met Tyr Ala Thr Thr Asn
               100             105             110

Trp Ser Met Met Lys Ala Val Asp Ala Ala Thr Gly Lys Leu Leu Trp
           115             120             125

Ser Tyr Asp Pro Arg Val Pro Gly Asn Ile Ala Asp Lys Gly Cys Cys
       130             135             140

Asp Thr Val Asn Arg Gly Ala Ala Tyr Trp Asn Gly Lys Val Tyr Phe
145             150             155             160

Gly Thr Phe Asp Gly Arg Leu Ile Ala Leu Asp Ala Lys Thr Gly Lys
               165             170             175

Leu Val Trp Ser Val Asn Thr Ile Pro Pro Glu Ala Glu Leu Gly Lys
               180             185             190

Gln Arg Ser Tyr Thr Val Asp Gly Ala Pro Arg Ile Ala Lys Gly Arg
       195             200             205

Val Ile Ile Gly Asn Gly Gly Ser Glu Phe Gly Ala Arg Gly Phe Val
       210             215             220

Ser Ala Phe Asp Ala Glu Thr Gly Lys Val Asp Trp Arg Phe Phe Thr
225             230             235             240

Val Pro Asn Pro Lys Asn Glu Pro Asp Ala Ala Ser Asp Ser Val Leu
               245             250             255

Met Asn Lys Ala Tyr Gln Thr Trp Ser Pro Thr Gly Ala Trp Thr Arg
               260             265             270

Gln Gly Gly Gly Gly Thr Val Trp Asp Ser Ile Val Tyr Asp Pro Val
       275             280             285

Ala Asp Leu Val Tyr Leu Gly Val Gly Asn Gly Ser Pro Trp Asn Tyr
       290             295             300
```

Lys Tyr Arg Ser Glu Gly Lys Gly Asp Asn Leu Phe Leu Gly Ser Ile
305   310   315   320

Val Ala Leu Lys Pro Glu Thr Gly Glu Tyr Val Trp His Phe Gln Glu
325   330   335

Thr Pro Met Asp Gln Trp Asp Phe Thr Ser Asp Gln Gln Ile Met Thr
340   345   350

Leu Asp Leu Pro Ile Asn Gly Glu Thr Arg His Val Ile Val His Ala
355   360   365

Arg Lys Asn Gly Phe Phe Tyr Ile Ile Asp Ala Lys Thr Gly Glu Phe
370   375   380

Ile Ser Gly Lys Asn Tyr Val Tyr Val Asn Trp Ala Ser Gly Leu Asp
385   390   395   400

Pro Lys Thr Gly Arg Pro Ile Tyr Asn Pro Asp Ala Leu Tyr Thr Leu
405   410   415

Thr Gly Lys Glu Trp Tyr Gly Ile Pro Gly Asp Leu Gly Gly His Asn
420   425   430

Phe Ala Ala Met Ala Phe Ser Pro Lys Thr Gly Leu Val Tyr Ile Pro
435   440   445

Ala Gln Gln Val Pro Phe Leu Tyr Thr Asn Gln Val Gly Gly Phe Thr
450   455   460

Pro His Pro Asp Ser Trp Asn Leu Gly Leu Asp Met Asn Lys Val Gly
465   470   475   480

Ile Pro Asp Ser Pro Glu Ala Lys Gln Ala Phe Val Lys Asp Leu Lys
485   490   495

Gly Trp Ile Val Ala Trp Asp Pro Gln Lys Gln Ala Glu Ala Trp Arg
500   505   510

Val Asp His Lys Gly Pro Trp Asn Gly Gly Ile Leu Ala Thr Gly Gly
515   520   525

Asp Leu Leu Phe Gln Gly Leu Ala Asn Gly Glu Phe His Ala Tyr Asp
530   535   540

Ala Thr Asn Gly Ser Asp Leu Phe His Phe Ala Ala Asp Ser Gly Ile
545   550   555   560

Ile Ala Pro Pro Val Thr Tyr Leu Ala Asn Gly Lys Gln Tyr Val Ala
565   570   575

Val Glu Val Gly Trp Gly Gly Ile Tyr Pro Phe Phe Leu Gly Gly Leu
580   585   590

Ala Arg Thr Ser Gly Trp Thr Val Asn His Ser Arg Ile Ile Ala Phe
595   600   605

Ser Leu Asp Gly Lys Ser Gly Pro Leu Pro Lys Gln Asn Asp Gln Gly
   610   615   620

Phe Leu Pro Val Lys Pro Pro Ala Gln Phe Asp Ser Lys Arg Thr Asp
625   630   635   640

Asn Gly Tyr Phe Gln Phe Gln Thr Tyr Cys Ala Ala Cys His Gly Asp
         645   650   655

Asn Ala Glu Gly Ala Gly Val Leu Pro Asp Leu Arg Trp Ser Gly Ser
         660   665   670

Ile Arg His Glu Asp Ala Phe Tyr Asn Val Val Gly Arg Gly Ala Leu
    675   680   685

Thr Ala Tyr Gly Met Asp Arg Leu His Gly Asn Met Asn Pro Thr Glu
   690   695   700

Ile Glu Asp Ile Arg Gln Phe Leu Ile Lys Arg Ala Asn Glu Thr Tyr
705   710   715   720

Gln Arg Glu Val Asp Ala Arg Lys Asn Ala Asp Gly Ile Pro Glu Gln
         725   730   735

Leu Pro
738

4. <u>SEQ ID NO: 4:</u>

    SEQUENCE TYPE: amino acid
    SEQUENCE LENGTH: 468
    MOLECULE TYPE: protein
    ORIGINAL SOURCE
    ORGANISM: Acetobacter altoacetigenes MH-24

Met Ile Asn Arg Leu Lys Val Thr Phe Ser Ala Ala Ala Phe Ser Leu
1   5   10   15

Leu Ala Gly Thr Ala Leu Ala Gln Thr Pro Asp Ala Asp Ser Ala Leu
                20   25   30

Val Gln Lys Gly Ala Tyr Val Ala Arg Leu Gly Asp Cys Val Ala Cys
        35   40   45

His Thr Ala Leu His Gly Gln Ser Tyr Ala Gly Gly Leu Glu Ile Lys
        50   55   60

Ser Pro Ile Gly Thr Ile Tyr Ser Thr Asn Ile Thr Pro Asp Pro Thr
65   70   75   80

Tyr Gly Ile Gly Arg Tyr Thr Phe Ala Glu Phe Asp Glu Ala Val Arg
                85   90   95

His Gly Ile Arg Lys Asp Gly Ser Thr Leu Tyr Pro Ala Met Pro Tyr
                100   105   110

Pro Ser Phe Ser Arg Met Thr Lys Glu Asp Met Gln Ala Leu Tyr Ala
        115   120   125

Tyr Phe Met His Gly Val Lys Pro Val Ala Gln Pro Asp Lys Gln Pro
        130   135   140

Asp Ile Ser Trp Pro Leu Ser Met Arg Trp Pro Leu Gly Ile Trp Arg
145   150   155   160

Met Met Phe Ser Pro Ser Pro Lys Asp Phe Thr Pro Ala Pro Gly Thr
                165   170   175

Asp Pro Glu Ile Ala Arg Gly Asp Tyr Leu Val Thr Gly Pro Gly His
                180   185   190

Cys Gly Ala Cys His Thr Pro Arg Gly Phe Ala Met Gln Glu Lys Ala
        195   200   205

Leu Asp Ala Ala Gly Gly Pro Asp Phe Leu Ser Gly Gly Ala Pro Ile
        210   215   220

Asp Asn Trp Val Ala Pro Ser Leu Arg Asn Asp Pro Val Val Gly Leu
225   230   235   240

Gly Arg Trp Ser Glu Asp Asp Ile Tyr Thr Phe Leu Lys Ser Gly Arg
                245   250   255

Ile Asp His Ser Ala Val Phe Gly Gly Met Gly Asp Val Val Ala Trp
                260   265   270

Ser Thr Gln Tyr Phe Thr Asp Asp Asp Leu His Ala Ile Ala Lys Tyr
        275   280   285

Leu Lys Ser Leu Pro Pro Val Pro Pro Ser Gln Gly Asn Tyr Thr Tyr
        290   295   300

18

```
Asp Pro Ser Thr Ala Asn Met Leu Ala Ser Gly Asn Thr Ala Ser Val
305             310         315         320

Pro Gly Ala Asp Thr Tyr Val Lys Glu Cys Ala Ile Cys His Arg Asn
            325         330             335

Asp Gly Gly Gly Val Ala Arg Met Phe Pro Pro Leu Ala Gly Asn Pro
        340             345             350

Val Val Val Thr Glu Asn Pro Thr Ser Leu Val Asn Val Ile Ala His
        355             360         365

Gly Gly Val Leu Pro Pro Ser Asn Trp Ala Pro Ser Ala Val Ala Met
    370             375             380

Pro Gly Tyr Ser Lys Ser Leu Ser Ala Gln Gln Ile Ala Asp Val Val
385             390         395             400

Asn Phe Ile Arg Thr Ser Trp Gly Asn Lys Ala Pro Gly Thr Val Thr
            405             410             415

Ala Ala Asp Val Thr Lys Leu Arg Asp Thr Gly Ala Pro Val Ser Ser
        420             425             430

Ser Gly Trp Asn Ser Val Ser Ser Gly Trp Ser Val Phe Leu Pro Gln
    435             440             445

Pro Tyr Gly Ser Gly Trp Thr Phe Ala Pro Gln Thr His Thr Gly Gln
    450             455             460

Asp Ala Ala Gln
465         468
```

Claims

1. A structural gene of the membrane-bound alcohol dehydrogenase complex having a size of about 7.0 kilo base being derived from a microorganism belonging to the genus <u>Acetobacter</u> and having the following restriction enzyme map:

```
PstI    ClaI    SmaIBamHI    BamHIClaISalISalI        PstI
```
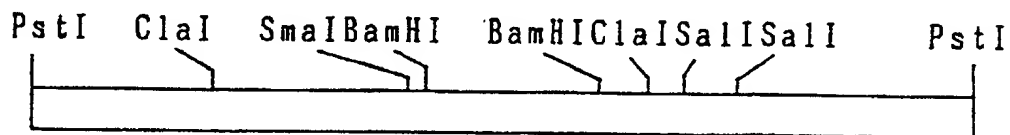
2. The structural gene according to claim 1, wherein said membrane-bound alcohol dehydrogenase complex is composed of proteins having a molecular weight of about 72,000 and a molecular weight of about 44,000.

3. The structural gene according to claim 2, encoding a protein with a molecular weight of about 72,000, and having the following nucleotide sequence:

```
        10        20        30        40      , 50
ATGATTTCTGCCGTTTTCGGAAAAAGACGTTCTCTGAGCAGAACGCTTACAGCCGGAACG

        70        80        90       100       110
ATATGTGCGGCTCTCATCTCCGGGTATGCCACCATGGCATCCGCAGATGACGGGCAGGGC

       130       140       150       160       170
GCCACGGGGGAAGCGATCATCCATGCCGATGATCACCCCGGTAACTGGATGACCTATGGC

       190       200       210       220       230
CGCACCTATTCTGACCAGCGCTACAGCCCGCTGGATCAGATCAACCGTTCCAATGTCGGT

       250       260       270       280       290
AACCTGAAGCTGGCCTGGTATCTGGACCTTGATACCAACCGTGGCCAGGAAGGCACGCCC

       310       320       330 ·     340       350
CTGGTTATTGATGGCGTCATGTACGCCACCACCAACTGGAGCATGATGAAAGCCGTCGAC

       370       380       390       400       410
GCCGCAACCGGCAAGCTGCTGTGGTCCTATGACCCGCGCGTGCCCGGCAACATTGCCGAC

       430       440       450       460       470
AAGGGCTGCTGTGACACGGTCAACCGTGGCGCGGCATACTGGAATGGCAAGGTCTATTTC

       490       500       510       520       530
GGCACGTTCGACGGTCGCCTGATCGCGCTGGACGCCAAGACCGGCAAGCTGGTCTGGAGC

       550       560       570       580       590
GTCAACACCATTCCGCCCGAAGCGGAACTGGGCAAGCAGCGTTCCTATACGGTTGACGGC

       610       620       630       640       650
GCGCCCGTATCGCCAAGGGCCGCGTGATCATCGGTAACGGTGGTTCCGAATTCGGTGCC

       670       680       690       700       710
CGTGGCTTCGTCAGCGCGTTCGATGCGGAAACCGGCAAGGTCGACTGGCGCTTCTTCACG

       730       740       750       760       770
GTTCCGAACCCCAAGAACGAACCGGACGCTGCATCCGACAGCGTGCTGATGAACAAGGCC

       790       800       810       820       830
TACCAGACCTGGAGCCCGACCGGCGCCTGGACCCGCCAGGGTGGCGGCGGCACGGTATGG

       850       860       870       880       890
GATTCCATCGTGTATGACCCCGTGGCCGACCTGGTCTACCTGGGCGTTGGCAACGGTTCG

       910       920       930       940       950
CCGTGGAACTACAAGTACCGTTCCGAAGGCAAGGGCGACAACCTGTTCCTGGGCAGCATC
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          970       980       990       1000      1010
     GTCGCACTGAAGCCGGAAACCGGCGAATACGTCTGGCATTTCCAGGAAACGCCGATGGAC

          1030      1040      1050      1060      1070
     CAGTGGGACTTCACCTCGGACCAGCAGATCATGACGCTTGACCTGCCGATCAATGGTGAA

          1090      1100      1110      1120      1130
     ACCCGCCACGTCATCGTCCATGCGCGCAAGAACGGCTTCTTCTACATCATCGATGCGAAG

          1150      1160      1170      1180      1190
     ACCGGTGAGTTCATCTCGGGCAAGAACTACGTCTATGTGAACTGGGCCAGCGGCCTTGAT

          1210      1220      1230      1240      1250
     CCCAAGACCGGCCGTCCGATCTACAACCCCGATGCGCTCTACACCCTTACGGGCAAGGAA

          1270      1280      1290      1300      1310
     TGGTACGGCATTCCGGGTGACCTTGGCGGCCATAACTTCGCGGCCATGGCGTTCAGCCCC

          1330      1340      1350      1360      1370
     AAGACCGGGCTGGTCTATATTCCGGCGCAGCAGGTTCCGTTCCTGTACACCAATCAGGTC

          1390      1400      1410      1420      1430
     GGTGGCTTCACGCCGCACCCCGACAGCTGGAACCTGGGTCTGGACATGAACAAGGTCGGT

          1450      1460      1470      1480      1490
     ATTCCCGACTCGCCTGAAGCCAAGCAGGCCTTCGTGAAGGACCTGAAGGGCTGGATCGTG

          1510      1520      1530      1540      1550
     GCCTGGGATCCGCAGAAGCAGGCTGAAGCATGGCGCGTGGACCACAAGGGGCCGTGGAAC

          1570      1580      1590      1600      1610
     GGCGGTATCCTGGCAACTGGCGGCGACCTGCTGTTCCAGGGCTTGGCGAACGGCGAATTC

          1630      1640      1650      1660      1670
     CATGCCTATGACGCGACGAACGGTTCCGACCTGTTCCACTTCGCGGCGGACAGCGGCATC

          1690      1700      1710      1720      1730
     ATCGCACCGCCTGTGACCTACCTTGCCAATGGCAAGCAGTATGTTGCGGTTGAAGTGGGC

          1750      1760      1770      1780      1790
     TGGGGCGGCATCTATCCGTTCTTCCTTGGTGGCCTGGCCCGTACCAGCGGCTGGACCGTC

          1810      1820      1830      1840      1850
     AACCACTCGCGCATCATTGCCTTCTCGCTCGATGGCAAGTCCGGCCCGCTGCCCAAGCAG

          1870      1880      1890      1900      1910
     AATGACCAGGGCTTCCTGCCCGTCAAGCCGCCGGCACAGTTCGACAGCAAGCGTACCGAT
```

```
        1930      1940      1950      1960      1970
AACGGTTACTTCCAGTTCCAGACCTATTGCGCCGCCTGTCATGGCGATAACGCAGAAGGT

        1990      2000      2010      2020      2030
GCCGGTGTGCTGCCTGACCTGCGCTGGTCCGGGTCCATCCGTCATGAGGACGCGTTCTAC

        2050      2060      2070      2080      2090
AATGTTGTCGGCCGCGGCGCGCTTACCGCCTACGGTATGGATCGCTTGCACGGTAACATG

        2110      2120      2130      2140      2150
AACCCGACCGAGATTGAGGACATCCGCCAGTTCCTGATCAAGCGTGCGAACGAGACCTAT

        2170      2180      2190      2200      2210
CAGAGGGAAGTTGATGCCCGGAAGAACGCTGACGGTATCCCCGAGCAGCTGCCG
```

4. The structural gene according to claim 2, encoding a protein with a molecular weight of about 44,000, and having the following nucleotide sequence:

```
                 10          20          30          40          50
ATGATCAACAGACTTAAGGTGACATTCAGCGCGGCAGCGTTTAGTCTGCTGGCAGGGACG

                 70          80          90         100         110
GCATTGGCACAGACGCCAGATGCTGACTCCGCGCTGGTCCAGAAGGGGGCATATGTCGCG

                130         140         150         160         170
CGACTGGGTGACTGCGTAGCATGTCATACCGCTCTCCATGGACAGTCGTACGCAGGCGGG

                190         200         210         220         230
CTTGAAATCAAGAGCCCGATCGGTACGATCTACTCCACGAACATCACACCGGACCCGACC

                250         260         270         280         290
TACGGTATCGGTCGCTACACCTTCGCCGAATTCGACGAAGCCGTGCGCCATGGTATCCGC

                310         320         330         340         350
AAGGACGGTTCCACGCTGTATCCGGCCATGCCGTATCCCTCCTTCTCGCGCATGACGAAG

                370         380         390         400         410
GAAGACATGCAGGCGCTGTATGCGTACTTCATGCATGGGGTGAAGCCGGTCGCGCAGCCG

                430         440         450         460         470
GACAAGCAGCCGGACATCTCCTGGCCCTTGTCCATGCGCTGGCCGCTGGGCATCTGGCGC

                490         500         510         520         530
ATGATGTTCTCGCCTTCGCCGAAGGACTTCACGCCGGCGCCAGGCACGGATCCTGAAATC

                550         560         570         580         590
GCACGTGGCGATTATCTGGTTACCGGCCCCGGGCATTGCGGTGCGTGTCATACCCCGCGT

                610         620         630         640         650
GGCTTCGCCATGCAGGAAAAGGCGCTGGACGCTGCCGGTGGTCCTGACTTCCTGTCCGGT

                670         680         690         700         710
GGCGCACCGATCGACAACTGGGTCGCGCCGAGCCTGCGCAACGATCCTGTCGTTGGTCTG

                730         740         750         760         770
GGCCGCTGGTCCGAGGATGACATCTACACCTTCCTGAAGTCCGGCCGTATCGACCACTCC

                790         800         810         820         830
GCCGTGTTCGGTGGCATGGGCGATGTGGTGGCATGGAGCACCCAGTACTTCACCGATGAC

                850         860         870         880         890
GACCTGCACGCCATCGCGAAGTACCTGAAGAGCCTGCCGCCGGTGCCGCCGTCACAGGGC

                910         920         930         940         950
AACTACACCTACGATCCGTCCACCGCGAACATGCTGGCTTCGGGTAATACCGCCAGCGTT
```

      970   980   990   1000   1010

CCGGGTGCTGATACGTATGTGAAGGAATGCGCCATCTGTCACCGTAACGACGGTGGTGGC

     1030   1040   1050   1060   1070

GTGGCCCGCATGTTCCCGCCGCTGGCTGGCAACCCGGTTGTCGTGACCGAGAACCCGACC

     1090   1100   1110   1120   1130

TCGCTGGTGAACGTGATTGCGCATGGTGGCGTGCTGCCGCCGAGCAACTGGGCACCGTCC

     1150   1160   1170   1180   1190

GCAGTGGCAATGCCGGGTTACAGCAAGTCGCTGTCCGCCCAGCAGATTGCTGATGTGGTC

     1210   1220   1230   1240   1250

AACTTCATCCGCACCAGCTGGGGCAACAAGGCGCCCGGCACCGTTACGGCTGCGGATGTT

     1270   1280   1290   1300   1310

ACCAAGCTGCGCGACACGGGCGCCCCGGTTTCCAGCTCTGGCTGGAACAGCGTGAGCAGC

     1330   1340   1350   1360   1370

GGCTGGTCGGTCTTCCTGCCGCAGCCTTACGGCTCGGGCTGGACGTTTGCCCCGCAGACG

     1390   1400

CACACCGGTCAGGACGCCGCACAG

5. The structural gene according to claim 2, encoding a protein with a molecular weight of about 72,000 and the following amino acid sequence:

<!-- 5 -->
```
                                          10                            20
        MetIleSerAlaValPheGlyLysArgArgSerLeuSerArgThrLeuThrAlaGlyThr
                                          30                            40
        IleCysAlaAlaLeuIleSerGlyTyrAlaThrMetAlaSerAlaAspAspGlyGlnGly
                                          50                            60
        AlaThrGlyGluAlaIleIleHisAlaAspAspHisProGlyAsnTrpMetThrTyrGly
                                          70                            80
        ArgThrTyrSerAspGlnArgTyrSerProLeuAspGlnIleAsnArgSerAsnValGly
                                          90                            100
        AsnLeuLysLeuAlaTrpTyrLeuAspLeuAspThrAsnArgGlyGlnGluGlyThrPro
                                          110                           120
        LeuValIleAspGlyValMetTyrAlaThrThrAsnTrpSerMetMetLysAlaValAsp
                                          130                           140
        AlaAlaThrGlyLysLeuLeuTrpSerTyrAspProArgValProGlyAsnIleAlaAsp
                                          150                           160
        LysGlyCysCysAspThrValAsnArgGlyAlaAlaTyrTrpAsnGlyLysValTyrPhe
                                          170                           180
        GlyThrPheAspGlyArgLeuIleAlaLeuAspAlaLysThrGlyLysLeuValTrpSer
                                          190                           200
        ValAsnThrIleProProGluAlaGluLeuGlyLysGlnArgSerTyrThrValAspGly
                                          210                           220
        AlaProArgIleAlaLysGlyArgValIleIleGlyAsnGlyGlySerGluPheGlyAla
                                          230                           240
        ArgGlyPheValSerAlaPheAspAlaGluThrGlyLysValAspTrpArgPhePheThr
                                          250                           260
        ValProAsnProLysAsnGluProAspAlaAlaSerAspSerValLeuMetAsnLysAla
                                          270                           280
        TyrGlnThrTrpSerProThrGlyAlaTrpThrArgGlnGlyGlyGlyGlyThrValTrp
                                          290                           300
        AspSerIleValTyrAspProValAlaAspLeuValTyrLeuGlyValGlyAsnGlySer
                                          310                           320
        ProTrpAsnTyrLysTyrArgSerGluGlyLysGlyAspAsnLeuPheLeuGlySerIle
                                          330                           340
        ValAlaLeuLysProGluThrGlyGluTyrValTrpHisPheGlnGluThrProMetAsp
                                          350                           360
        GlnTrpAspPheThrSerAspGlnGlnIleMetThrLeuAspLeuProIleAsnGlyGlu
                                          370                           380
        ThrArgHisValIleValHisAlaArgLysAsnGlyPhePheTyrIleIleAspAlaLys
                                          390                           400
        ThrGlyGluPheIleSerGlyLysAsnTyrValTyrValAsnTrpAlaSerGlyLeuAsp
```

<!-- 55 -->

25

```
                    410                          420
ProLysThrGlyArgProIleTyrAsnProAspAlaLeuTyrThrLeuThrGlyLysGlu
                    430                          440
TrpTyrGlyIleProGlyAspLeuGlyGlyHisAsnPheAlaAlaMetAlaPheSerPro
                    450                          460
LysThrGlyLeuValTyrIleProAlaGlnGlnValProPheLeuTyrThrAsnGlnVal
                    470                          480
GlyGlyPheThrProHisProAspSerTrpAsnLeuGlyLeuAspMetAsnLysValGly
                    490                          500
IleProAspSerProGluAlaLysGlnAlaPheValLysAspLeuLysGlyTrpIleVal
                    510                          520
AlaTrpAspProGlnLysGlnAlaGluAlaTrpArgValAspHisLysGlyProTrpAsn
                    530                          540
GlyGlyIleLeuAlaThrGlyGlyAspLeuLeuPheGlnGlyLeuAlaAsnGlyGluPhe
                    550                          560
HisAlaTyrAspAlaThrAsnGlySerAspLeuPheHisPheAlaAlaAspSerGlyIle
                    570                          580
IleAlaProProValThrTyrLeuAlaAsnGlyLysGlnTyrValAlaValGluValGly
                    590                          600
TrpGlyGlyIleTyrProPhePheLeuGlyGlyLeuAlaArgThrSerGlyTrpThrVal
                    610                          620
AsnHisSerArgIleIleAlaPheSerLeuAspGlyLysSerGlyProLeuProLysGln
                    630                          640
AsnAspGlnGlyPheLeuProValLysProProAlaGlnPheAspSerLysArgThrAsp
                    650                          660
AsnGlyTyrPheGlnPheGlnThrTyrCysAlaAlaCysHisGlyAspAsnAlaGluGly
                    670                          680
AlaGlyValLeuProAspLeuArgTrpSerGlySerIleArgHisGluAspAlaPheTyr
                    690                          700
AsnValValGlyArgGlyAlaLeuThrAlaTyrGlyMetAspArgLeuHisGlyAsnMet
                    710                          720
AsnProThrGluIleGluAspIleArgGlnPheLeuIleLysArgAlaAsnGluThrTyr
                    730
GlnArgGluValAspAlaArgLysAsnAlaAspGlyIleProGluGlnLeuPro
```

6. The structural gene according to claim 2, encoding a protein having a molecular weight of about 44,000 and the following amino acid sequence:

```
                           10                                    20
MetIleAsnArgLeuLysValThrPheSerAlaAlaAlaPheSerLeuLeuAlaGlyThr
                           30                                    40
AlaLeuAlaGlnThrProAspAlaAspSerAlaLeuValGlnLysGlyAlaTyrValAla
                           50                                    60
ArgLeuGlyAspCysValAlaCysHisThrAlaLeuHisGlyGlnSerTyrAlaGlyGly
                           70                                    80
LeuGluIleLysSerProIleGlyThrIleTyrSerThrAsnIleThrProAspProThr
                           90                                   100
TyrGlyIleGlyArgTyrThrPheAlaGluPheAspGluAlaValArgHisGlyIleArg
                          110                                   120
LysAspGlySerThrLeuTyrProAlaMetProTyrProSerPheSerArgMetThrLys
                          130                                   140
GluAspMetGlnAlaLeuTyrAlaTyrPheMetHisGlyValLysProValAlaGlnPro
                          150                                   160
AspLysGlnProAspIleSerTrpProLeuSerMetArgTrpProLeuGlyIleTrpArg
                          170                                   180
MetMetPheSerProSerProLysAspPheThrProAlaProGlyThrAspProGluIle
                          190                                   200
AlaArgGlyAspTyrLeuValThrGlyProGlyHisCysGlyAlaCysHisThrProArg
```

27

```
                          210                        220
      GlyPheAlaMetGlnGluLysAlaLeuAspAlaAlaGlyGlyProAspPheLeuSerGly
                          230                        240
      GlyAlaProIleAspAsnTrpValAlaProSerLeuArgAsnAspProValValGlyLeu
                          250                        260
      GlyArgTrpSerGluAspAspIleTyrThrPheLeuLysSerGlyArgIleAspHisSer
                          270                        280
      AlaValPheGlyGlyMetGlyAspValValAlaTrpSerThrGlnTyrPheThrAspAsp
                          290                        300
      AspLeuHisAlaIleAlaLysTyrLeuLysSerLeuProProValProProSerGlnGly
                          310                        320
      AsnTyrThrTyrAspProSerThrAlaAsnMetLeuAlaSerGlyAsnThrAlaSerVal
                          330                        340
      ProGlyAlaAspThrTyrValLysGluCysAlaIleCysHisArgAsnAspGlyGlyGly
                          350                        360
      ValAlaArgMetPheProProLeuAlaGlyAsnProValValValThrGluAsnProThr
                          370                        380
      SerLeuValAsnValIleAlaHisGlyGlyValLeuProProSerAsnTrpAlaProSer
                          390                        400
      AlaValAlaMetProGlyTyrSerLysSerLeuSerAlaGlnGlnIleAlaAspValVal


                          410                        420
      AsnPheIleArgThrSerTrpGlyAsnLysAlaProGlyThrValThrAlaAlaAspVal
                          430                        440
      ThrLysLeuArgAspThrGlyAlaProValSerSerSerGlyTrpAsnSerValSerSer
                          450                        460
      GlyTrpSerValPheLeuProGlnProTyrGlySerGlyTrpThrPheAlaProGlnThr


      HisThrGlyGlnAspAlaAlaGln
```

7. A plasmid containing a structural gene according to any one of claims 1 to 6.

8. An acetic acid bacterium belonging to the genus <u>Acetobacter</u> or the genus <u>Gluconobacter</u> transformed with a plasmid according to claim 7.

9. A process for the preparation of a membrane-bound alcohol-dehydrogenase complex wherein an acetic acid bacterium according to claim 8 is cultivated under suitable conditions.

10. The process according to claim 9, additionally comprising the step of isolating said alcohol-dehydrogenase complex.
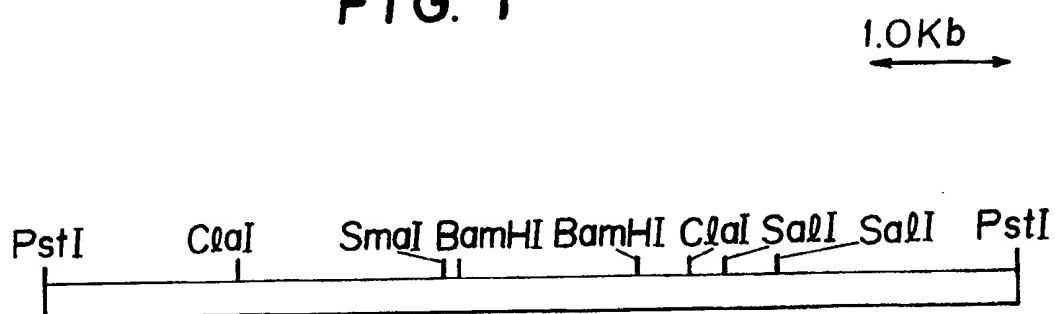
FIG. 1

1.0Kb →

PstI   ClaI   SmaI BamHI BamHI ClaI SalI SalI   PstI

FIG. 2

1.0Kb ↔

SmaI BamHI BamHI ClaI SalI SalI PstI SmaI

FIG. 3-1

```
          10         20         30         40         50
ATGATTTCTGCCGTTTTCGGAAAAAGACGTTCTCTGAGCAGAACGCTTACAGCCGGAACG
MetIleSerAlaValPheGlyLysArgArgSerLeuSerArgThrLeuThrAlaGlyThr

          70         80         90        100        110
ATATGTGCGGCTCTCATCTCCGGGTATGCCACCATGGCATCCGCAGATGACGGGCAGGGC
IleCysAlaAlaLeuIleSerGlyTyrAlaThrMetAlaSerAlaAspAspGlyGlnGly

         130        140        150        160        170
GCCACGGGGGAAGCGATCATCCATGCCGATGATCACCCCGGTAACTGGATGACCTATGGC
AlaThrGlyGluAlaIleIleHisAlaAspAspHisProGlyAsnTrpMetThrTyrGly

         190        200        210        220        230
CGCACCTATTCTGACCAGCGCTACAGCCCGCTGGATCAGATCAACCGTTCCAATGTCGGT
ArgThrTyrSerAspGlnArgTyrSerProLeuAspGlnIleAsnArgSerAsnValGly

         250        260        270        280        290
AACCTGAAGCTGGCCTGGTATCTGGACCTTGATACCAACCGTGGCCAGGAAGGCACGCCC
AsnLeuLysLeuAlaTrpTyrLeuAspLeuAspThrAsnArgGlyGlnGluGlyThrPro

         310        320        330        340        350
CTGGTTATTGATGGCGTCATGTACGCCACCACCAACTGGAGCATGATGAAAGCCGTCGAC
LeuValIleAspGlyValMetTyrAlaThrThrAsnTrpSerMetMetLysAlaValAsp

         370        380        390        400        410
GCCGCAACCGGCAAGCTGCTGTGGTCCTATGACCCGCGCGTGCCCGGCAACATTGCCGAC
AlaAlaThrGlyLysLeuLeuTrpSerTyrAspProArgValProGlyAsnIleAlaAsp

         430        440        450        460        470
AAGGGCTGCTGTGACACGGTCAACCGTGGCGCGGCATACTGGAATGGCAAGGTCTATTTC
LysGlyCysCysAspThrValAsnArgGlyAlaAlaTyrTrpAsnGlyLysValTyrPhe

         490        500        510        520        530
GGCACGTTCGACGGTCGCCTGATCGCGCTGGACGCCAAGACCGGCAAGCTGGTCTGGAGC
GlyThrPheAspGlyArgLeuIleAlaLeuAspAlaLysThrGlyLysLeuValTrpSer

         550        560        570        580        590
GTCAACACCATTCCGCCCGAAGCGGAACTGGGCAAGCAGCGTTCCTATACGGTTGACGGC
ValAsnThrIleProProGluAlaGluLeuGlyLysGlnArgSerTyrThrValAspGly
```
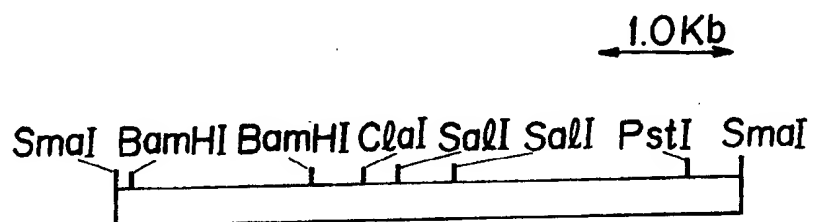
FIG. 3-2

```
         610       620       630       640       650
GCGCCCCGTATCGCCAAGGGCCGCGTGATCATCGGTAACGGTGGTTCCGAATTCGGTGCC
AlaProArgIleAlaLysGlyArgValIleIleGlyAsnGlyGlySerGluPheGlyAla
         670       680       690       700       710
CGTGGCTTCGTCAGCGCGTTCGATGCGGAAACCGGCAAGGTCGACTGGCGCTTCTTCACG
ArgGlyPheValSerAlaPheAspAlaGluThrGlyLysValAspTrpArgPhePheThr
         730       740       750       760       770
GTTCCGAACCCCAAGAACGAACCGGACGCTGCATCCGACAGCGTGCTGATGAACAAGGCC
ValProAsnProLysAsnGluProAspAlaAlaSerAspSerValLeuMetAsnLysAla
         790       800       810       820       830
TACCAGACCTGGAGCCCGACCGGCGCCTGGACCCGCCAGGGTGGCGGCGGCACGGTATGG
TyrGlnThrTrpSerProThrGlyAlaTrpThrArgGlnGlyGlyGlyGlyThrValTrp
         850       860       870       880       890
GATTCCATCGTGTATGACCCCGTGGCCGACCTGGTCTACCTGGGCGTTGGCAACGGTTCG
AspSerIleValTyrAspProValAlaAspLeuValTyrLeuGlyValGlyAsnGlySer
         910       920       930       940       950
CCGTGGAACTACAAGTACCGTTCCGAAGGCAAGGGCGACAACCTGTTCCTGGGCAGCATC
ProTrpAsnTyrLysTyrArgSerGluGlyLysGlyAspAsnLeuPheLeuGlySerIle
         970       980       990       1000      1010
GTCGCACTGAAGCCGGAAACCGGCGAATACGTCTGGCATTTCCAGGAAACGCCGATGGAC
ValAlaLeuLysProGluThrGlyGluTyrValTrpHisPheGlnGluThrProMetAsp
         1030      1040      1050      1060      1070
CAGTGGGACTTCACCTCGGACCAGCAGATCATGACGCTTGACCTGCCGATCAATGGTGAA
GlnTrpAspPheThrSerAspGlnGlnIleMetThrLeuAspLeuProIleAsnGlyGlu
         1090      1100      1110      1120      1130
ACCCGCCACGTCATCGTCCATGCGCGCAAGAACGGCTTCTTCTACATCATCGATGCGAAG
ThrArgHisValIleValHisAlaArgLysAsnGlyPhePheTyrIleIleAspAlaLys
         1150      1160      1170      1180      1190
ACCGGTGAGTTCATCTCGGGCAAGAACTACGTCTATGTGAACTGGGCCAGCGGCCTTGAT
ThrGlyGluPheIleSerGlyLysAsnTyrValTyrValAsnTrpAlaSerGlyLeuAsp
```

FIG. 3-3

```
          1210        1220        1230        1240        1250
CCCAAGACCGGCCGTCCGATCTACAACCCCGATGCGCTCTACACCCTTACGGGCAAGGAA
ProLysThrGlyArgProIleTyrAsnProAspAlaLeuTyrThrLeuThrGlyLysGlu
          1270        1280        1290        1300        1310
TGGTACGGCATTCCGGGTGACCTTGGCGGCCATAACTTCGCGGCCATGGCGTTCAGCCCC
TrpTyrGlyIleProGlyAspLeuGlyGlyHisAsnPheAlaAlaMetAlaPheSerPro
          1330        1340        1350        1360        1370
AAGACCGGGCTGGTCTATATTCCGGCGCAGCAGGTTCCGTTCCTGTACACCAATCAGGTC
LysThrGlyLeuValTyrIleProAlaGlnGlnValProPheLeuTyrThrAsnGlnVal
          1390        1400        1410        1420        1430
GGTGGCTTCACGCCGCACCCCGACAGCTGGAACCTGGGTCTGGACATGAACAAGGTCGGT
GlyGlyPheThrProHisProAspSerTrpAsnLeuGlyLeuAspMetAsnLysValGly
          1450        1460        1470        1480        1490
ATTCCCGACTCGCCTGAAGCCAAGCAGGCCTTCGTGAAGGACCTGAAGGGCTGGATCGTG
IleProAspSerProGluAlaLysGlnAlaPheValLysAspLeuLysGlyTrpIleVal
          1510        1520        1530        1540        1550
GCCTGGGATCCGCAGAAGCAGGCTGAAGCATGGCGCGTGGACCACAAGGGGCCGTGGAAC
AlaTrpAspProGlnLysGlnAlaGluAlaTrpArgValAspHisLysGlyProTrpAsn
          1570        1580        1590        1600        1610
GGCGGTATCCTGGCAACTGGCGGCGACCTGCTGTTCCAGGGCTTGGCGAACGGCGAATTC
GlyGlyIleLeuAlaThrGlyGlyAspLeuLeuPheGlnGlyLeuAlaAsnGlyGluPhe
          1630        1640        1650        1660        1670
CATGCCTATGACGCGACGAACGGTTCCGACCTGTTCCACTTCGCGGCGGACAGCGGCATC
HisAlaTyrAspAlaThrAsnGlySerAspLeuPheHisPheAlaAlaAspSerGlyIle
          1690        1700        1710        1720        1730
ATCGCACCGCCTGTGACCTACCTTGCCAATGGCAAGCAGTATGTTGCGGTTGAAGTGGGC
IleAlaProProValThrTyrLeuAlaAsnGlyLysGlnTyrValAlaValGluValGly
          1750        1760        1770        1780        1790
TGGGGCGGCATCTATCCGTTCTTCCTTGGTGGCCTGGCCCCGTACCAGCGGCTGGACCGTC
TrpGlyGlyIleTyrProPhePheLeuGlyGlyLeuAlaArgThrSerGlyTrpThrVal
```

FIG.3-4

```
           1810        1820        1830        1840        1850
AACCACTCGCGCATCATTGCCTTCTCGCTCGATGGCAAGTCCGGCCCGCTGCCCAAGCAG
AsnHisSerArgIleIleAlaPheSerLeuAspGlyLysSerGlyProLeuProLysGln
           1870        1880        1890        1900        1910
AATGACCAGGGCTTCCTGCCCGTCAAGCCGCCGGCACAGTTCGACAGCAAGCGTACCGAT
AsnAspGlnGlyPheLeuProValLysProProAlaGlnPheAspSerLysArgThrAsp
           1930        1940        1950        1960        1970
AACGGTTACTTCCAGTTCCAGACCTATTGCGCCGCCTGTCATGGCGATAACGCAGAAGGT
AsnGlyTyrPheGlnPheGlnThrTyrCysAlaAlaCysHisGlyAspAsnAlaGluGly
           1990        2000        2010        2020        2030
GCCGGTGTGCTGCCTGACCTGCGCTGGTCCGGGTCCATCCGTCATGAGGACGCGTTCTAC
AlaGlyValLeuProAspLeuArgTrpSerGlySerIleArgHisGluAspAlaPheTyr
           2050        2060        2070        2080        2090
AATGTTGTCGGCCGCGGCGCGCTTACCGCCTACGGTATGGATCGCTTGCACGGTAACATG
AsnValValGlyArgGlyAlaLeuThrAlaTyrGlyMetAspArgLeuHisGlyAsnMet
           2110        2120        2130        2140        2150
AACCCGACCGAGATTGAGGACATCCGCCAGTTCCTGATCAAGCGTGCGAACGAGACCTAT
AsnProThrGluIleGluAspIleArgGlnPheLeuIleLysArgAlaAsnGluThrTyr
           2170        2180        2190        2200        2210
CAGAGGGAAGTTGATGCCCGGAAGAACGCTGACGGTATCCCCGAGCAGCTGCCG
GlnArgGluValAspAlaArgLysAsnAlaAspGlyIleProGluGlnLeuPro
```

FIG. 4-1

```
          10        20        30        40        50
ATGATCAACAGACTTAAGGTGACATTCAGCGCGGCAGCGTTTAGTCTGCTGGCAGGGACG
MetIleAsnArgLeuLysValThrPheSerAlaAlaAlaPheSerLeuLeuAlaGlyThr
          70        80        90       100       110
GCATTGGCACAGACGCCAGATGCTGACTCCGCGCTGGTCCAGAAGGGGGCATATGTCGCG
AlaLeuAlaGlnThrProAspAlaAspSerAlaLeuValGlnLysGlyAlaTyrValAla
         130       140       150       160       170
CGACTGGGTGACTGCGTAGCATGTCATACCGCTCTCCATGGACAGTCGTACGCAGGCGGG
ArgLeuGlyAspCysValAlaCysHisThrAlaLeuHisGlyGlnSerTyrAlaGlyGly
         190       200       210       220       230
CTTGAAATCAAGAGCCCGATCGGTACGATCTACTCCACGAACATCACACCGGACCCGACC
LeuGluIleLysSerProIleGlyThrIleTyrSerThrAsnIleThrProAspProThr
         250       260       270       280       290
TACGGTATCGGTCGCTACACCTTCGCCGAATTCGACGAAGCCGTGCGCCATGGTATCCGC
TyrGlyIleGlyArgTyrThrPheAlaGluPheAspGluAlaValArgHisGlyIleArg
         310       320       330       340       350
AAGGACGGTTCCACGCTGTATCCGGCCATGCCGTATCCCTCCTTCTCGCGCATGACGAAG
LysAspGlySerThrLeuTyrProAlaMetProTyrProSerPheSerArgMetThrLys
         370       380       390       400       410
GAAGACATGCAGGCGCTGTATGCGTACTTCATGCATGGGGTGAAGCCGGTCGCGCAGCCG
GluAspMetGlnAlaLeuTyrAlaTyrPheMetHisGlyValLysProValAlaGlnPro
         430       440       450       460       470
GACAAGCAGCCGGACATCTCCTGGCCCTTGTCCATGCGCTGGCCGCTGGGCATCTGGCGC
AspLysGlnProAspIleSerTrpProLeuSerMetArgTrpProLeuGlyIleTrpArg
         490       500       510       520       530
ATGATGTTCTCGCCTTCGCCGAAGGACTTCACGCCGGCGCCAGGCACGGATCCTGAAATC
MetMetPheSerProSerProLysAspPheThrProAlaProGlyThrAspProGluIle
         550       560       570       580       590
GCACGTGGCGATTATCTGGTTACCGGCCCCGGGCATTGCGGTGCGTGTCATACCCCGCGT
AlaArgGlyAspTyrLeuValThrGlyProGlyHisCysGlyAlaCysHisThrProArg
```

FIG. 4-2

```
        610       620       630       640       650
GGCTTCGCCATGCAGGAAAAGGCGCTGGACGCTGCCGGTGGTCCTGACTTCCTGTCCGGT
GlyPheAlaMetGlnGluLysAlaLeuAspAlaAlaGlyGlyProAspPheLeuSerGly
        670       680       690       700       710
GGCGCACCGATCGACAACTGGGTCGCGCCGAGCCTGCGCAACGATCCTGTCGTTGGTCTG
GlyAlaProIleAspAsnTrpValAlaProSerLeuArgAsnAspProValValGlyLeu
        730       740       750       760       770
GGCCGCTGGTCCGAGGATGACATCTACACCTTCCTGAAGTCCGGCCGTATCGACCACTCC
GlyArgTrpSerGluAspAspIleTyrThrPheLeuLysSerGlyArgIleAspHisSer
        790       800       810       820       830
GCCGTGTTCGGTGGCATGGGCGATGTGGTGGCATGGAGCACCCAGTACTTCACCGATGAC
AlaValPheGlyGlyMetGlyAspValValAlaTrpSerThrGlnTyrPheThrAspAsp
        850       860       870       880       890
GACCTGCACGCCATCGCGAAGTACCTGAAGAGCCTGCCGCCGGTGCCGCCGTCACAGGGC
AspLeuHisAlaIleAlaLysTyrLeuLysSerLeuProProValProProSerGlnGly
        910       920       930       940       950
AACTACACCTACGATCCGTCCACCGCGAACATGCTGGCTTCGGGTAATACCGCCAGCGTT
AsnTyrThrTyrAspProSerThrAlaAsnMetLeuAlaSerGlyAsnThrAlaSerVal
        970       980       990       1000      1010
CCGGGTGCTGATACGTATGTGAAGGAATGCGCCATCTGTCACCGTAACGACGGTGGTGGC
ProGlyAlaAspThrTyrValLysGluCysAlaIleCysHisArgAsnAspGlyGlyGly
        1030      1040      1050      1060      1070
GTGGCCCGCATGTTCCCGCCGCTGGCTGGCAACCCGGTTGTCGTGACCGAGAACCCGACC
ValAlaArgMetPheProProLeuAlaGlyAsnProValValValThrGluAsnProThr
        1090      1100      1110      1120      1130
TCGCTGGTGAACGTGATTGCGCATGGTGGCGTGCTGCCGCCGAGCAACTGGGCACCGTCC
SerLeuValAsnValIleAlaHisGlyGlyValLeuProProSerAsnTrpAlaProSer
        1150      1160      1170      1180      1190
GCAGTGGCAATGCCGGGTTACAGCAAGTCGCTGTCCGCCCAGCAGATTGCTGATGTGGTC
AlaValAlaMetProGlyTyrSerLysSerLeuSerAlaGlnGlnIleAlaAspValVal
```

FIG. 4-3

```
          1210      1220      1230      1240      1250
AACTTCATCCGCACCAGCTGGGGCAACAAGGCGCCCGGCACCGTTACGGCTGCGGATGTT
AsnPheIleArgThrSerTrpGlyAsnLysAlaProGlyThrValThrAlaAlaAspVal
          1270      1280      1290      1300      1310
ACCAAGCTGCGCGACACGGGCGCCCCGGTTTCCAGCTCTGGCTGGAACAGCGTGAGCAGC
ThrLysLeuArgAspThrGlyAlaProValSerSerSerGlyTrpAsnSerValSerSer
          1330      1340      1350      1360      1370
GGCTGGTCGGTCTTCCTGCCGCAGCCTTACGGCTCGGGCTGGACGTTTGCCCCGCAGACG
GlyTrpSerValPheLeuProGlnProTyrGlySerGlyTrpThrPheAlaProGlnThr
          1390      1400
CACACCGGTCAGGACGCCGCACAG
HisThrGlyGlnAspAlaAlaGln
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